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Susceptibility of Porcine Intestine to Pilus-Mediated Adhesion by Some Isolates of Piliated Enterotoxigenic *Escherichia coli* Increases with Age

BELA NAGY,† THOMAS A. CASEY, SHANNON C. WHIPP,* AND HARLEY W. MOON

Physiopathology Research Unit, National Animal Disease Center, USDA Agricultural Research Service, Ames, Iowa 50010

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Two porcine isolates of enterotoxigenic *Escherichia coli* (ETEC) (serogroup O157 and O141) derived from fatal cases of postweaning diarrhea and lacking K88, K99, F41, and 987P pili (4P⁻ ETEC) were tested for adhesiveness to small-intestinal epithelia of pigs of different ages. Neither strain adhered to isolated intestinal brush borders of newborn (1-day-old) pigs in the presence of mannose. However, mannose-resistant adhesion occurred when brush borders from 10-day- and 3- and 6-week-old pigs were used. Electron microscopy revealed that both strains produced fine (3.5-nm) and type 1 pili at 37°C but only type 1 pili at 18°C. Mannose-resistant in vitro adhesion to brush borders of older pigs correlated with the presence of fine pili. These strains produced predominantly fine pili in ligated intestinal loops of both older and newborn pigs, but adherence was greater in loops in older pigs. Immunoelectron microscopic studies, using antiserum raised against piliated bacteria and absorbed with nonpiliated bacteria, of samples from brush border adherence studies revealed labelled appendages between adherent bacteria and intestinal microvilli. Orogastric inoculation of pigs weaned at 10 and 21 days of age indicated significantly ($P < 0.001$) higher levels of adhesion by the ETEC to the ileal epithelia of older pigs than to that of younger ones. We suggest that small-intestinal adhesion and colonization by these ETEC isolates is dependent on receptors that develop progressively with age during the first 3 weeks after birth. Furthermore, our data are consistent with the hypothesis that the fine pili described mediate intestinal adhesion by the 4P⁻ ETEC strains studied.

Enterotoxic colibacillosis of newborn pigs has been studied extensively. Three enterotoxins (LT, STaP, and STb) and four pilus antigens (K88, K99, 987P, F41 [also named F4, F5, F6, and F41]) are recognized as important in pathogenesis of the disease (11, 24, 31). Enterotoxic colibacillosis also occurs in recently weaned pigs (typically at 3 to 6 weeks of age). Data from the United States indicate that more than one half of the enterotoxigenic *Escherichia coli* (ETEC) strains in both age groups are K88⁺ (31, 32). Most of the K88⁻ ETEC strains from neonatal pigs produce pilus antigen K99 or 987P (18), both of which facilitate adhesion and colonization in neonatal pigs.

In contrast, K88⁻ ETEC strains from postweaning diarrhea have been less extensively studied. Sporadic occurrence of K99⁺ ETEC among postweaning isolates has been reported in the United States and Sweden (30, 31), and such strains seem to occur more frequently in Japan (23). However, ETEC strains producing pilus antigens K99, 987P, and F41 are usually not isolated from pigs with postweaning diarrhea (31). This may be explained by age-related resistance to adhesion and colonization by strains of these pilus types. For example, older pigs become resistant to K99-mediated adhesion because of the loss of receptors (27). Age-related resistance to 987P is associated with an increase of 987P receptors in the mucus in the intestinal lumen that apparently inhibit adhesion and colonization by ETEC in older pigs (6).

We surveyed K88⁻ ETEC isolates from fatal cases of postweaning diarrhea in swine and found that only 1 of 51 carries a known porcine ETEC adhesin (21). We use the term 4P⁻ ETEC to designate pathogenic porcine ETEC strains that do not produce K88, K99, F41, or 987P. Pathogenicity studies revealed that some of these strains could colonize the small intestine and produce diarrhea in weaned pigs (4a). Colonization by the 4P⁻ ETEC strains was characterized by preferential adhesion of the bacteria to the villi covering Peyer's patches and by filamentous appendages between bacteria and microvilli (22a).

We hypothesized that novel pili (previously unrecognized) mediate adhesion and colonization of 4P⁻ ETEC strains in older pigs after weaning. We further hypothesized that the susceptibility of pig intestine to adhesion and colonization by such strains of ETEC increases with age during the first 3 weeks after birth. To test these hypotheses, we investigated in vitro adhesiveness of 4P⁻ ETEC strains to isolated brush borders from newborn and older pigs in relation to their piliation. Furthermore, we used ligated intestinal loop and orogastric inoculation experiments to determine if age-dependent variation in colonization and adhesion occurs.

MATERIALS AND METHODS

Bacterial strains. *E. coli* 2134 (O157:H19) and 2171 (O141:H4), both hemolytic and STaP and STb enterotoxin producers, were isolated from the small intestines of weaned pigs that died as a result of diarrhea. Both strains were from the collection of ETEC reported earlier to lack K88, K99, 987P,

* Corresponding author.
† Permanent address: Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary.

and F41 pili (21). *E. coli Bam* (3), *E. coli* 123 (a nonenterotoxigenic *E. coli* strain) (20), and *E. coli* 2041 (O157, K88) served as controls.

Preparation of antisera. Antisera against the pili of 4P$^-$ ETEC strain 2134 (anti-2134P sera) were raised (2, 22) in rabbits injected intravenously either with 6-h tryptic soy broth (TSB) cultures of *E. coli* 2134 grown at 37°C or with bacteria grown overnight on MINCA-Is agar (12) at 37°C. Sera were absorbed against the same bacteria grown at 18°C. In preliminary studies, absorbed anti-2134P sera gave a strong cross-reaction (agglutination and fluorescence) with 4P$^-$ ETEC strain 2171 (4a). Both anti-2134P sera were used for agglutination at a 1:100 dilution. The anti-type 1 pilus serum, which was produced by Dominick et al. (9) by using purified pili of *E. coli* O78:K80, agglutinated *E. coli Bam* at a dilution of 1:200. It was used at a dilution of 1:10 to 1:100 for slide agglutination and at a dilution of 1:100 for immunofluorescence.

Animal experiments. Pigs of different ages, obtained from one herd, were used in these experiments. The producer vaccinated the pregnant gilts and sows with a bacterin (Litterguard; Norden Laboratories) containing K88, K99, F41, and 987P according to the manufacturer's instructions.

Ligated ileal loops (10 cm) were prepared (at 0.2, 0.5, and 1.0 m proximal from the ileocecal valve), as described earlier (19), in neonatal (<48-h-old), 10-day-old, and several-week-old pigs, respectively. There were eight pigs in each group. Postsurgical discomfort of the pigs was minimized by the intramuscular administration of butorphanol tartarate (Torbugesic; Aveco Co., Fort Dodge, Iowa) (0.1 ml/kg of body weight) and by providing additional heat. One-day-old pigs had access to colostrum for 5 to 9 h after birth. They were brought into the laboratory, fasted, and given 50 ml of normal swine serum intraperitoneally 30 h before surgery. Loops were inoculated with 10^8 bacteria of fresh overnight TSB cultures diluted in phosphate-buffered saline (PBS). Pigs were euthanatized, and loops were examined 18 h after inoculation. Segments (50 cm) of small intestine adjacent, but anterior, to the ileal loop segments were removed for brush border preparations.

Orogastric inoculation of weaned pigs was performed as described previously (28) by using 10^{10} bacteria per pig. Pigs were weaned directly from the farm into isolation units. One half of the pigs in each of nine litters were weaned at 10 days of age; the other half were weaned at 21 days of age and delivered on the day of weaning. Approximately equal numbers of pigs from each litter were assigned to experimental and control groups (10 to 12 pigs in each group), placed into isolation, and kept and fed ad lib as described before (28). Orogastric inoculation via a stomach tube was done on day 3 after weaning. Pigs were weighed and observed daily. One half of each group were euthanatized on day 2 postinoculation; the other half were euthanatized on day 3 postinoculation. Ileal samples for histopathology, immunofluorescence studies, and electron microscopy were processed as described below.

Statistical comparisons were made by using the Student *t* test.

In vitro adherence. In vitro adhesion tests utilizing isolated brush borders were performed as described previously (7, 29), except that 0.5 ml of washed bacteria (approximately 10^9/ml) was added to an equal amount of brush border suspension (containing approximately 10^6 brush borders per ml), and incubated with gentle shaking at 37°C for 30 min. The bacterium-brush border suspension was washed by centrifugation (200 × *g*) and resuspended in PBS three times.

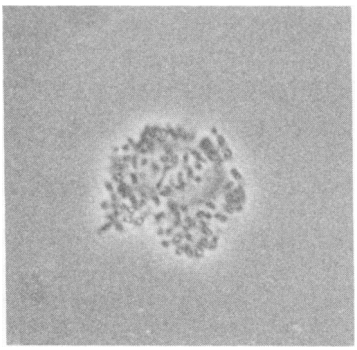

FIG. 1. In vitro adherence of 4P$^-$ ETEC to intestinal brush border membranes of a 6-week-old pig.

The final pellet was resuspended in 0.3 ml of PBS, and the average number of adherent bacteria-brush border was estimated on the basis of phase-contrast microscopy of the first 20 bacterium-brush border complexes. Any numbers estimated to be above 40 were recorded as 40. In vitro adhesion tests were also performed with 0.5% D-mannose in the bacterium-brush border suspension.

Histopathology, immunofluorescence, and electron microscopy. Tissue samples from intestinal loops were processed for histopathology (hematoxylin-and-eosin-stained sections), for indirect fluorescent-antibody (IFA) studies, and for transmission electron microscopy (22). Association indexes (AIs) were determined as described previously (2), except that only IF was done to determine contiguity of bacteria to epithelial cells (scored 1 to 5) and the extent of fluorescing bacterial layers (scored 1 to 5). The two values were multiplied to give the AI. Samples of intestinal mucosa from loops inoculated with strain 2134 were fixed with glutaraldehyde and prepared for electron microscopy. Additional samples were fixed and stained with a paraformaldehyde-glutaraldehyde-ruthenium red solution as described previously (25). Bacteria were stained with 2% phosphotungstic acid for negative-contrast electron microscopy.

TABLE 1. In vitro adhesion and piliation of *E. coli* 2134, 2171, and *Bam*[a]

Strain	Culture[b]	In vitro adhesion[c]		Piliation[d]			
		Standard	D-Mannose	Electron microscopy		Agglutination	
				Type 1	Fine	Type 1	2134P
2134	37°C	++	++	+	+	+/−	++
	18°C	−	NT	+	−	−	−
2171	37°C	++	++	+	+	+/−	++
	18°C	+	−	+	−	−	−
Bam	37°C	+	−	+	−	+	−
	18°C	+	−	+	−	+	−

[a] *Bam*, Prototype strain for type 1 pili.
[b] 37°C, 18-h TSB cultures grown at 37°C; 18°C, 2- to 10-day-old TSB cultures grown at 18°C.
[c] To brush borders from 6-week-old pigs. NT, not tested.
[d] Agglutinations were done by using absorbed pilus antisera (anti-type 1 or anti-2134P) produced in rabbits against whole bacteria grown at 37°C and absorbed with bacteria grown at 18°C.

FIG. 2. Negatively stained electron micrograph showing type 1 pili and fine (approximately 3.5-nm) pili (arrows) of 4P$^-$ ETEC strain 2134 from tryptic soy broth culture grown at 37°C. Bar, 0.1 μm.

IEM. Immunoelectron microscopy (IEM) was performed on sections of bacterium-brush border complexes.

The absorbed anti-2134P serum (used in a 1:10 dilution) was mixed with the bacterium-brush border complexes and incubated with gentle rotation at 37°C for 30 min and then incubated overnight at 4°C and subsequently washed three times in PBS (containing 1% bovine serum albumin) by centrifugation. Anti-rabbit immunoglobulin labelled with 5-nm gold particles (Jansen Life Sciences Products) was used as the secondary antibody (dilution, 1:10). The gold-labelled secondary antibodies were added to the resuspended washed sediment, incubated at 37°C for 30 min, and washed, and the last sediment was embedded and processed for thin sections as described previously (22).

The procedure of Knutton et al. was followed (15) for IEM of crude extracts (13) of strain 2134 grown at 37°C on MINCA-Is agar and grown at 16°C in TSB by using the absorbed anti-2134P serum in a 1:100 dilution. Each sample

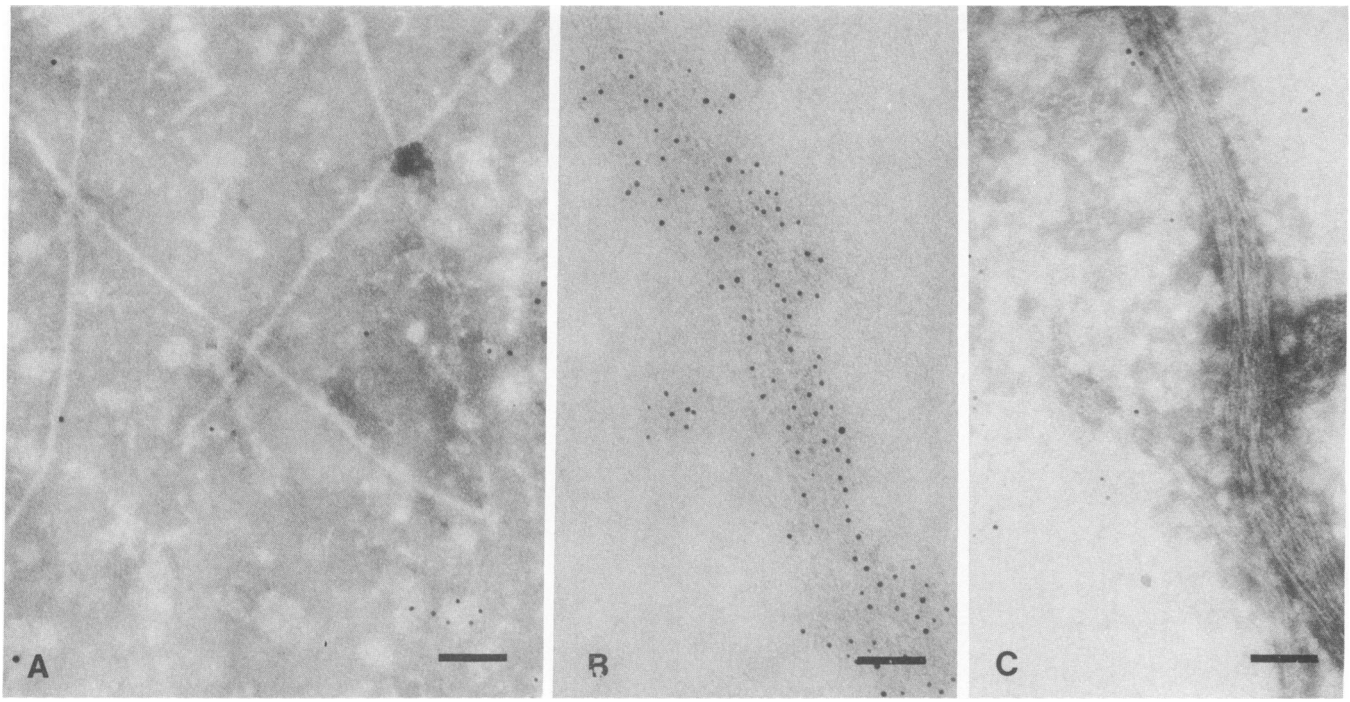

FIG. 3. IEM of the type 1 and fine pili of 4P$^-$ ETEC 2134. (A) The immunogold label is not associated with type 1 pili. This demonstrates that type 1 pili do not react with absorbed anti-2134P serum. (B) The immunogold label is associated with fine pili, demonstrating that fine pili react strongly with anti-2134P serum. (C) Fine pili do not react with normal rabbit serum. Bars, 0.1 μm.

was also examined by using normal rabbit serum (in the same dilution as the absorbed anti-2134P serum) as a negative control.

RESULTS

In vitro adhesion and piliation of 4P$^-$ ETEC. Large numbers of adherent bacteria of strains 2134 and 2171 were observed when brush borders from 6-week-old pigs were tested with bacteria grown at 37°C. This adhesion was characterized by formation of large clumps of bacteria on and around the brush borders. These clumps frequently covered the brush borders completely. In such cases, only a few or no brush borders remained free of bacteria. Some of these clumps involved several brush borders surrounded by the bacteria, forming large complexes of bacteria and brush borders (Fig. 1). The bacteria also formed aggregates (clumps) without obvious association to the brush border. This adhesiveness of both 4P$^-$ ETEC strains was resistant to D-mannose and disappeared when strain 2134 was grown at 18°C. However, cultures of strain 2171 grown at 18°C were adherent, and this adhesion was blocked by D-mannose (Table 1).

Newborn pig brush borders did not adhere to the two 4P$^-$ ETEC strains when bacteria were grown at 37°C, and the bacteria did not form large aggregates (clumps) in the presence of brush borders from newborn pigs. However, strain 2171 adhered to brush borders of neonates in a mannose-sensitive manner when cultures grown at 18°C were tested (data not shown). D-Mannose blocked the adhesion of the type 1 pilus control strain *Bam* to brush borders from both newborn and older pigs. Type 1 pilus serum inconsistently agglutinated both 4P$^-$ ETEC strains. Agglutination of the 4P$^-$ ETEC strains in absorbed anti-2134P serum occurred only when the bacteria were grown at 37°C (Table 1). Heat treatment (15 min, 100°C) eliminated agglutinability of both 4P$^-$ ETEC strains grown at 37°C (data not shown) and also eliminated in vitro adherence.

Electron microscopic examination of TSB cultures grown at 37°C revealed thick, type 1-like pili (referred to as type 1) and 3.5-nm pili (referred to as fine pili) on bacterial cells of both 4P$^-$ ETEC strains (Fig. 2). Only the type 1 pili were seen when several-day-old cultures grown at 18°C were examined. At this temperature, strain 2171 produced more type 1 pili than did strain 2134. The type 1 control strain *Bam* produced type 1 pili at both temperatures (Table 1).

The type 1 and fine pili of 4P$^-$ ETEC 2134 were also tested in crude extracts for their reaction to absorbed anti-2134P serum by using IEM with negative staining. This absorbed serum specifically reacted with the fine pili but did not react with the type 1 pili of strain 2134 (Fig. 3A to C).

IEM of in vitro adherent bacteria with absorbed anti-2134P serum revealed labelled appendages that were aggregated filamentous structures bridging the spaces between bacterial cell walls and host microvillus membranes (Fig. 4A). When the same samples were treated with normal rabbit serum, these structures were not labelled (Fig. 4B). Bacteria tended to adhere to the microvillus side of the brush border rather than to the nonmicrovillus side.

In vivo adhesion and piliation. Results of intestinal loop experiments indicated that adhesion of strain 2134 to the ileal epithelium increased with the age of pigs between 1 and 21 days. The AI, an indicator of in vivo adhesiveness, was consistently higher for 4P$^-$ ETEC 2134 than that for nonenterotoxigenic *E. coli* 123, and this difference was greater ($P < 0.05$) at 10, 21, and 42 days of age than at 1 day of age (Fig. 5). Moreover, there was a significant ($P < 0.05$) increase in

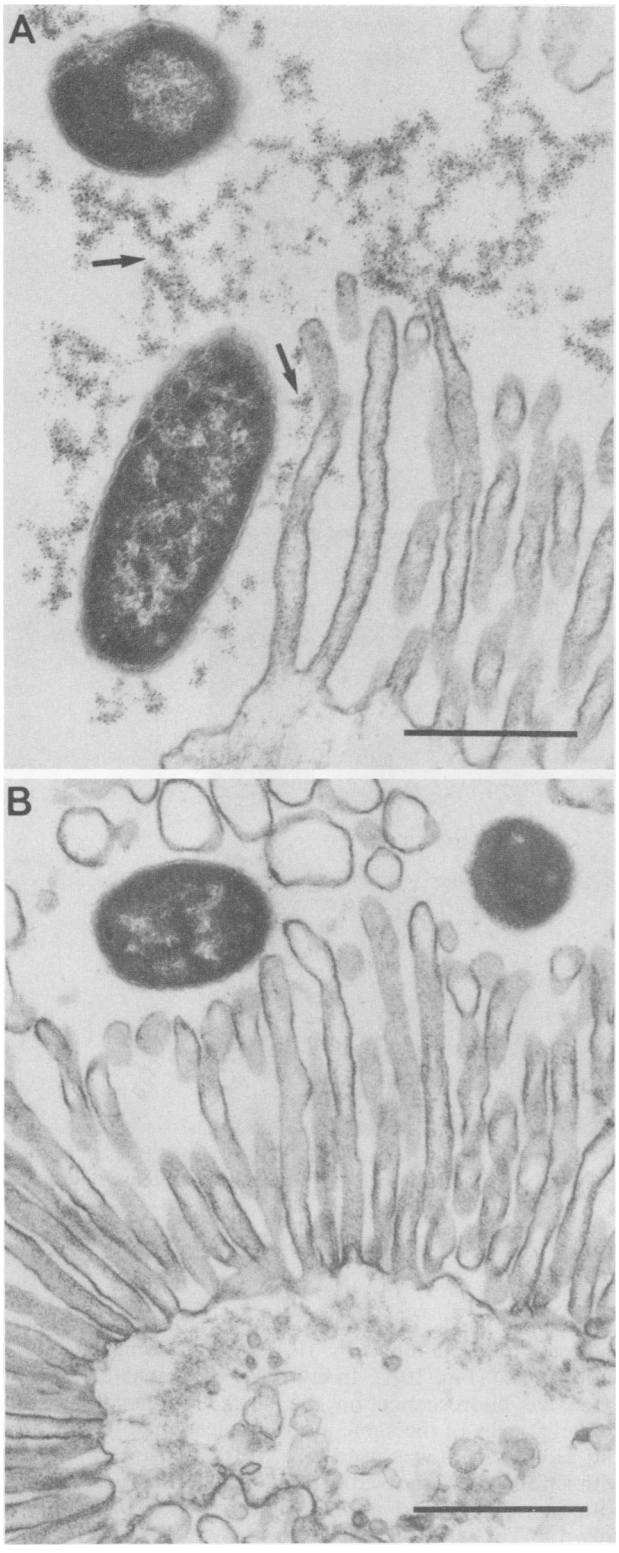

FIG. 4. Transmission electron micrographs of sections of in vitro adherent 4P⁻ ETEC strain 2134 to isolated intestinal brush borders. (A) Bacterial cells have irregular arrays of immunogold arising from their surfaces and bridging the spaces between bacteria and host microvillus membranes (arrows). In vitro bacterium-brush border complexes were first reacted with absorbed rabbit antiserum against pili of strain 2134 and then with anti-rabbit gold-labeled antibodies. (B) A normal rabbit serum-treated control sample showing no gold-labeled appendages. Bars, 1.0 μm.

the in vivo adhesiveness of strain 2134 when AIs in 2134-inoculated loops of 1- and 21-day-old pigs were compared. These trends were also evident when AIs of 1-day-old pigs were compared with those of 10- and 42-day-old pigs, but these differences did not reach significance. Strain 2134 formed only a few or no adherent microcolonies in loops of neonatal pigs (Fig. 6A). In contrast, in ileal loops of older pigs, strain 2134 formed patchy layers on the lateral surfaces of villi (Fig. 6B). Adhesion occurred more frequently and adherent layers were more extensive on the villi covering Peyer's patches (without preference for M cells) than on other areas of the mucosa. The K88⁺ ETEC strain 2041 adhered and produced high AIs in loops in six of eight newborn pigs and in six of eight 3-week-old pigs tested (mean AIs for K88-sensitive pigs, 20.4 and 18.6, respectively, for newborn and 3-week-old pigs).

Brush borders prepared from pigs showed a gradual increase of in vitro adhesiveness, i.e., larger numbers of adherent bacteria on a higher proportion of the brush borders, and a gradual increase of frequency and size of clumps from day 1 (no adhesion) to day 21 (Fig. 7).

Because we were concerned about piluslike artifacts resulting from capsular polysaccharides, in addition to performing glutaraldehyde fixation, we also fixed and stained intestinal-wall samples with ruthenium red to visualize polysaccharide material. Electron microscopy of mucosa from ileal loops inoculated with and colonized by the 4P⁻ ETEC strain 2134 revealed similar filamentous appendages between bacteria and intact microvilli when either procedure was used, without an indication of bacterial capsular polysaccharide (Fig. 8).

Negative-staining electron microscopy of samples from

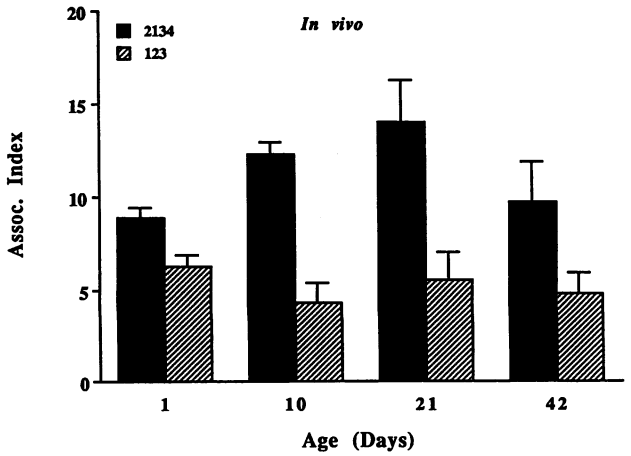

FIG. 5. In vivo adhesion (AI ± standard deviation) of 4P⁻ ETEC strain 2134 and the nonenteropathogenic control *E. coli* 123 in intestinal loops of pigs of different ages.

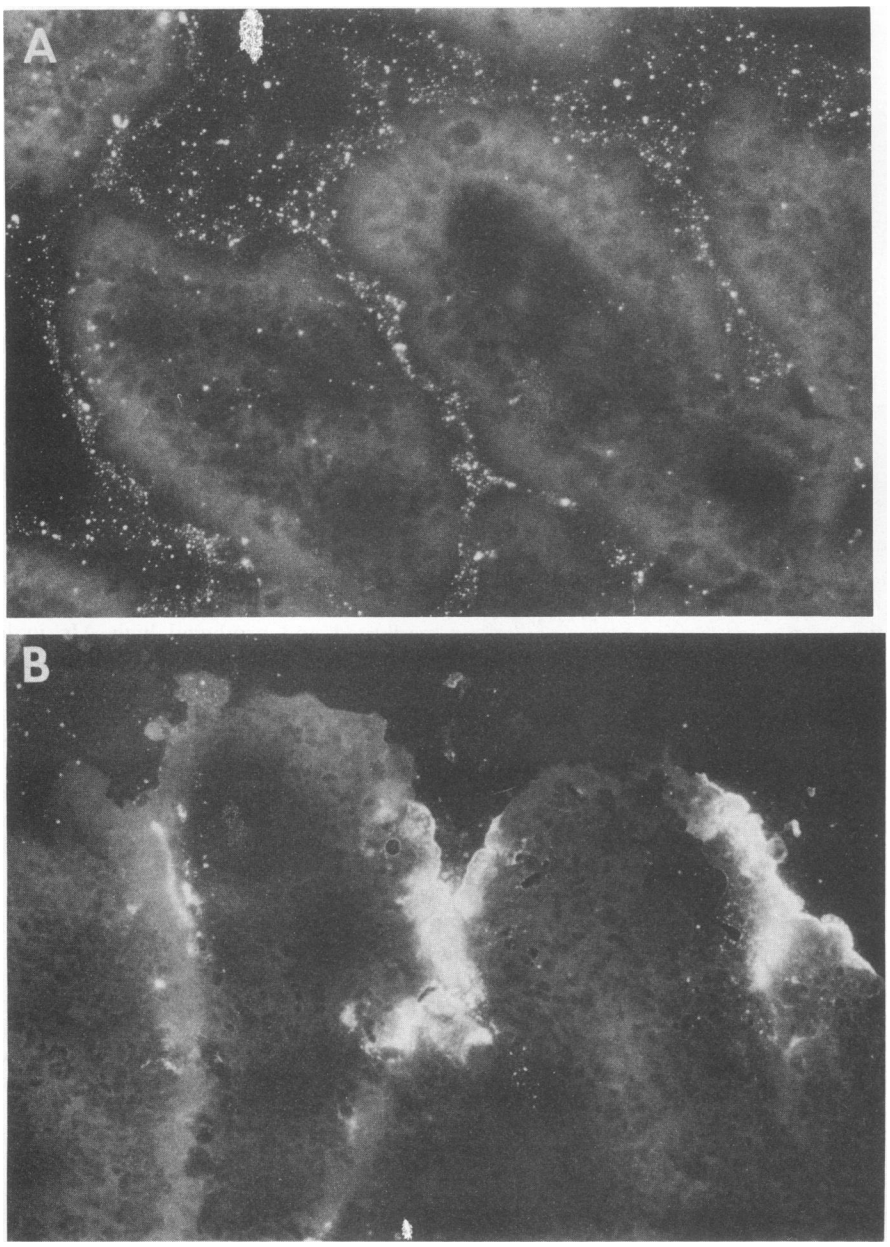

FIG. 6. IFA of frozen sections of intestinal loops of pigs of different ages 18 h after inoculation with 4P⁻ ETEC strain 2134. (A) Little or no adhesion of bacteria to epithelial cells in a newborn pig, with bacteria randomly distributed in mucus; (B) patches of intense fluorescence representing bacteria adherent to epithelial cells in 6-week-old pig.

ileal loops inoculated with strain 2134 revealed bacteria carrying fine pili in newborn, 21-day-old, and 6-week-old pigs (Fig. 9). Fine pili were more frequently observed than type 1 pili in the loop contents of three pigs from each age group (50 to 70% versus 0 to 20% of bacteria examined). The production of both kinds of pili by strain 2134 in vivo was also examined by IFA of frozen sections of ileal loops from newborn (n = 3) and 21-day-old (n = 3) pigs. In newborn pigs, no fluorescence was observed in sections of ileal loops when anti-type 1 pilus serum was used, but there was a spotty but bright fluorescence (as expected because of the small number of adherent bacteria present) in the same ileal samples when the absorbed anti-2134P serum was used. In 21-day-old pigs, in vivo production of type 1 pili examined by IFA showed some pale or no fluorescence (Fig. 10A). In contrast, there was a bright and extensive fluorescence on the surface of small-intestinal epithelial cells of the same loop samples when the absorbed anti-2134P serum was used (Fig. 10B). In vitro IFA studies with strain 2134 showed strong, bright fluorescence with both anti-type 1 and anti-2134P sera when bacteria were grown in TSB at 37°C, but only anti-type 1 serum produced similar, bright fluorescence when bacteria were grown in TSB at 18°C.

Orogastric inoculation of pigs (weaned at 10 or 21 days of age) with 4P⁻ ETEC strain 2134 resulted in a more intensive

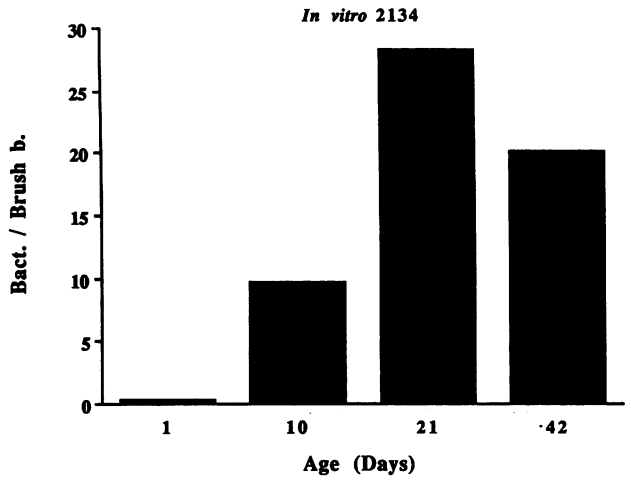

FIG. 7. Degree of in vitro adhesion of 4P⁻ ETEC strain 2134 to intestinal brush border membranes (eight pigs per age group).

adhesion of these bacteria to the ileal walls of older pigs than to those of younger pigs (Table 2). The difference between the AIs was highly significant ($P < 0.001$). Tests of in vitro adhesion of strain 2134 to brush border membranes from pigs in the two age groups also showed increased adhesiveness to brush borders from older pigs (Table 2). One day after infection, the mean relative weight gains of 10-day-old pigs were +0.59% for the nonenterotoxigenic *E. coli* 123-inoculated control and +0.67% for the 4P⁻ ETEC strain 2134-inoculated principal pigs. In contrast, the mean relative weight gains of 21-day-old pigs were +1.55% for the controls and −2.98% for the principals. The mean difference between controls and principals in the 10-day-old group was 0.26% (standard deviation = 4.42). In the 21-day-old group, it was 4.46% (standard deviation = 3.38). This last value represented a significant ($P < 0.05$) difference, and it was significantly ($P < 0.001$) higher than the difference between controls and principals in the 10-day-old group. Thus, the older pigs gained much less than the younger ones as a result of strain 2134 challenge.

DISCUSSION

We have demonstrated in vitro and in vivo adhesion to porcine intestine by postweaning porcine 4P⁻ ETEC strains. Adhesion increased with the age of the pigs between 1 and 21 days. We also report evidence consistent with the hypothesis that the responsible adhesive factors were fine pili.

ETEC carrying K99 or 987P pili adhere to a greater extent to intestinal epithelial cells of younger pigs than to intestinal epithelial cells of older pigs (8, 27) and are usually associated with disease in neonatal pigs (31). In contrast to such age-related resistance, pigs became more susceptible, rather than more resistant, to strains 2134 and 2171 with age. The exact reason for increased susceptibility with age was not determined by these studies.

The lower sensitivity of 1-day-old pig intestinal loops to adhesion by 4P⁻ ETEC strain 2134 could have been caused by residual colostral antibodies. However, the control K88⁺ ETEC (against which the sows were vaccinated) was not inhibited from adhesion under the same conditions. Furthermore, in vitro tests with isolated, washed brush

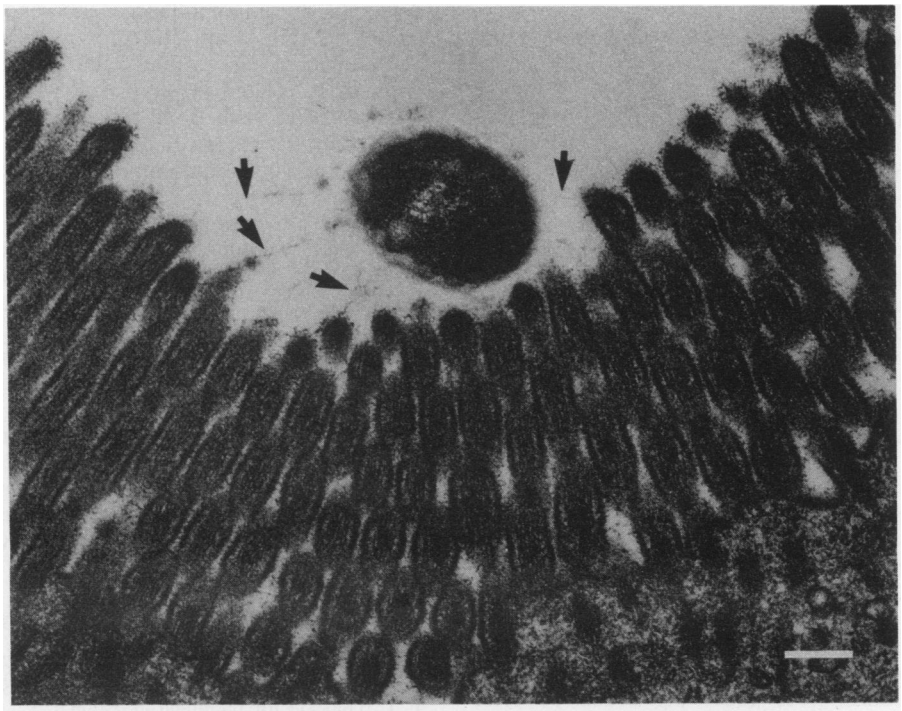

FIG. 8. Transmission electron micrograph of epithelial-cell microvilli and a bacterial cell in an ileal loop from a 6-week-old pig 18 h after inoculation with 4P⁻ ETEC strain 2134. Filamentous appendages extend from the bacterial surface to microvillous membranes of the epithelial cell (arrows). Bar, 0.2 μm.

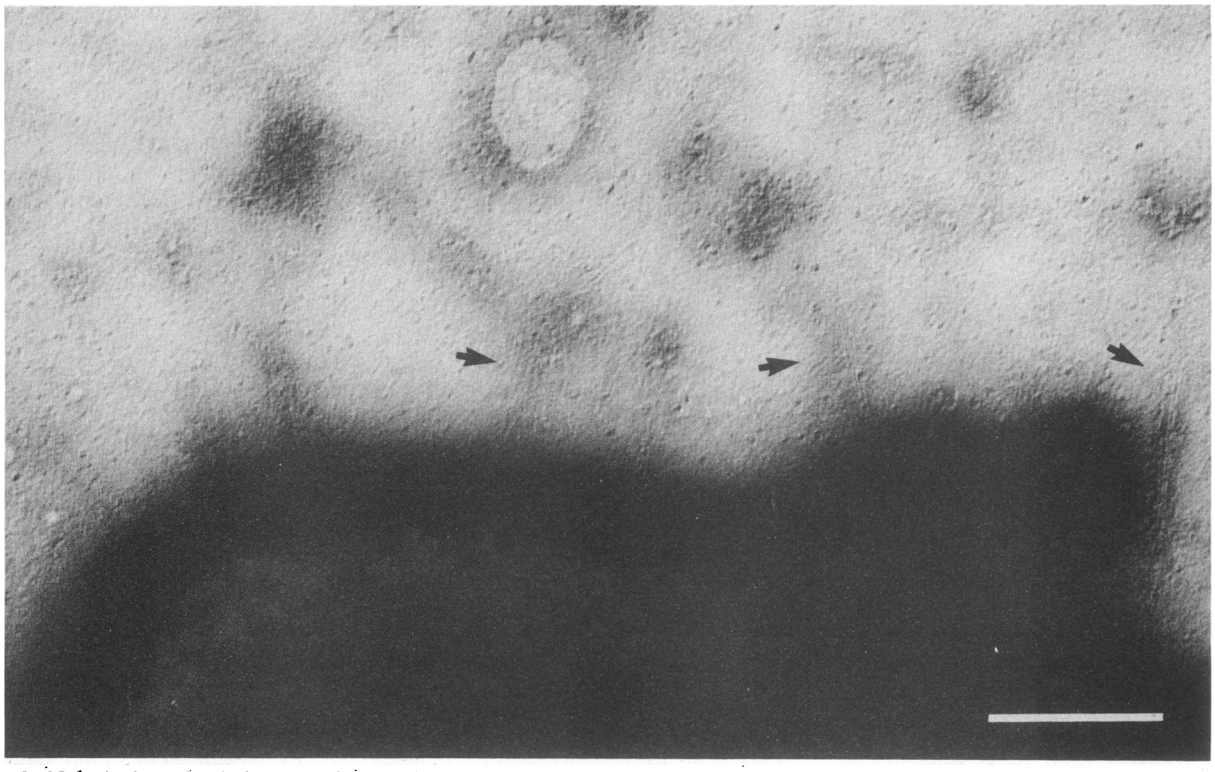

FIG. 9. Negatively stained electron micrograph showing only fine (approximately 3.5-nm) pili on the surface of 4P$^-$ ETEC strain 2134 grown in vivo (in loop, 18 h after inoculation of a 6-week-old pig). Bar, 0.2 μm.

borders from the same pigs were also nonadhesive, indicating that the lack of adhesion was not caused by colostral antibodies.

The increased sensitivity of weaned pigs to adhesion and colonization by postweaning ETEC isolates is reminiscent of the susceptibility of weaned rabbits to the enteropathogenic *E. coli* RDEC-1 (that only adheres to brush borders of rabbits of >21 days of age) and to some postweaning rabbit field isolates (4, 26). The preferential adhesion of these porcine 4P$^-$ ETEC strains to the Peyer's patches, however, differs from that of the rabbit enteropathogenic *E. coli* RDEC-1 (4, 5) for which the first sites of colonization are also the Peyer's patches. However, RDEC-1 colonizes the M cells as the primary site, where further bacterial growth results in colonization of the ileum and diarrhea. Our strains colonized the absorptive epithelial cells above Peyer's patches without preference to the M cells. In further contrast to these rabbit strains (4), our 4P$^-$ ETEC strains do not produce lesions characteristic of attaching-effacing *E. coli* (22a).

The 4P$^-$ ETEC strains produced both type 1 and fine pili. The following observations support the hypothesis that the fine pili, and not type 1 pili, were responsible for adherence. (i) The absorbed anti-2134 serum does not recognize type 1 pili, as determined by using IEM. (ii) Immunofluorescence and electron microscopy indicated that fine pili were abundant on bacteria grown at 37°C but not on bacteria grown at 18°C. (iii) The presence of fine pili correlated with mannose-resistant in vitro adhesion to older pig brush borders, whereas bacteria grown at 18°C did not adhere to older pig brush borders in the presence of mannose. In this respect, the adhesin of the 4P$^-$ bacteria showed the same mannose-resistant pattern as that of all other known porcine ETEC-specific adhesins (11). (iv) Fine pili were the predominant pili detectable by immunofluorescence and by electron microscopy on ETEC strain 2134 grown in vivo.

The in vivo expression of fine pili of strain 2134 did not seem to be related to pig age; it was equally detectable in 1-day-old and 3-week-old pig loops. Therefore, it could be concluded that receptors for this adhesin were less available on the small-intestinal epithelium of newborn pigs than on that from older pigs.

The fine pili demonstrated were antigenically distinct from pili already recognized as colonization factors of porcine ETEC (K88, K99, 987P, and F41). However, they may be related to other pili such as the one recently reported to be present on an edema disease strain (1) or on postweaning diarrhea strains (14). Their relationship to other animal *E. coli* pili such as FY (17), F165 (10), and F42 (16) remains to be investigated.

At this point, however, it can be concluded that the two 4P$^-$ ETEC strains adhere to the microvilli of older pigs significantly better than they do to microvilli of newborn pigs both in vitro and in vivo. Furthermore, their in vivo colonizing ability is significantly better in 3-week-old pigs than in 10-day-old pigs.

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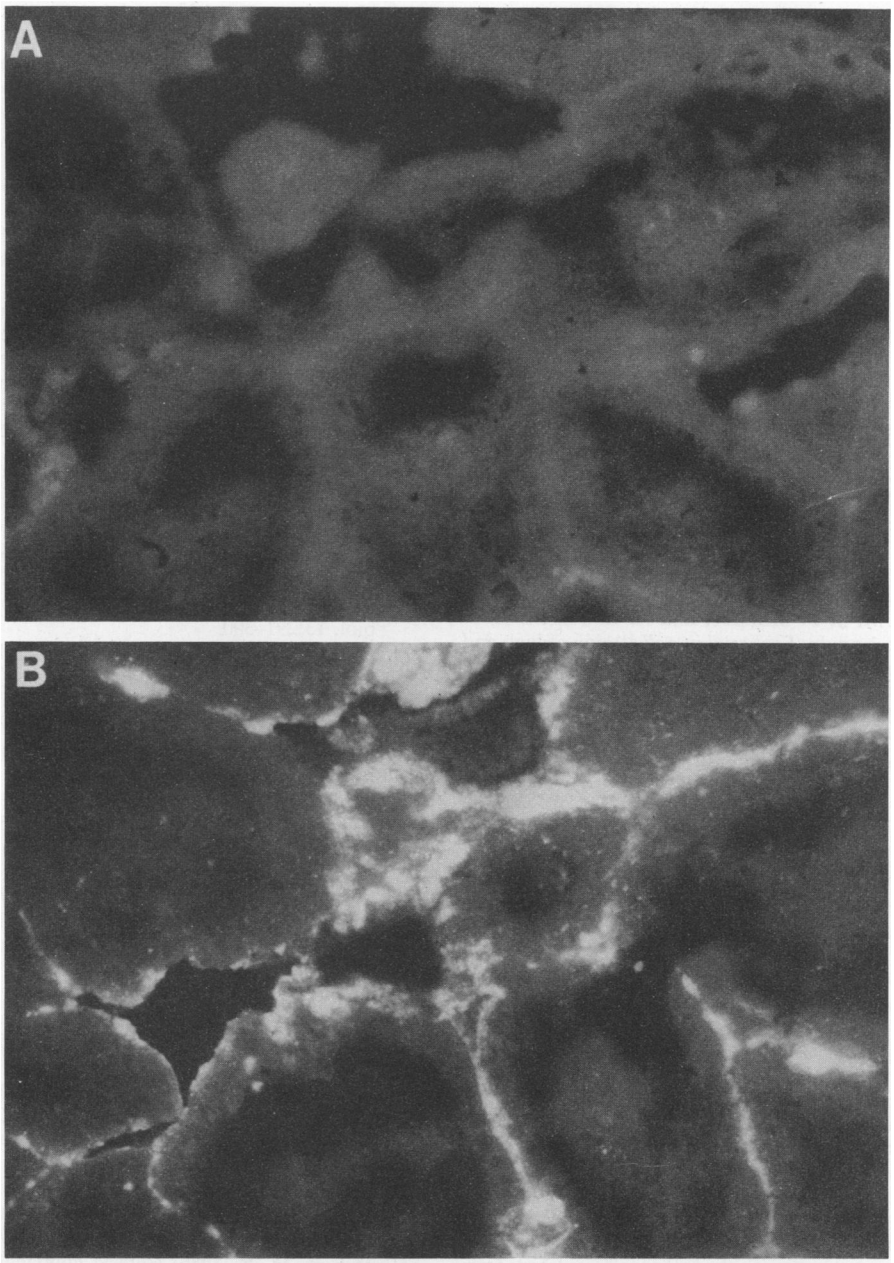

FIG. 10. IFA of frozen sections of ileal loop of a 3-week-old pig 18 h after inoculation with 4P⁻ ETEC strain 2134. (A) No fluorescence is observed in a section conjugated with anti-type 1 pilus serum; (B) intensely fluorescent bacterial layer covers the villi in a subsequent section of the same loop conjugated with anti-2134P serum.

TABLE 2. In vivo and in vitro adhesion of 4P⁻ ETEC strain 2134 to intestinal epithelia of pigs weaned at 10 and 21 days of age and inoculated 3 days postweaning

Age group (days)	In vivo		In vitro	
	Bacterial layers[a]	AI[b]	Adherence	Bact/BB[c]
10	7/12	11.8 ± 2.0	14/16[d]	9.7
21	11/11	23.7 ± 0.6	12/12	24.2

[a] Number of pigs with adherent bacterial layers demonstrated via histopathology (hematoxylin-and-eosin stain)/number of pigs tested.
[b] Mean ± standard deviation determined on the basis of all animals tested.
[c] Mean number of adherent bacteria per brush border.
[d] Bacteria did not adhere to brush borders from two pigs in this age group.

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